REMARKS

Prior to the present response, claims 1-55 were pending. By the present communication, no claims have been added or cancelled, and claims 1, 13, 26, 39, 46, 54 and 55 have been amended to define Applicants' invention with greater particularity. The amendments add no new matter, being fully supported by the Specification and original claims. Accordingly, claims 1-55 are currently pending in this application.

The Sequence Listing

Applicants respectfully traverse the assertion in the Office Action that this application fails to comply with the requirements of 37 C.F.R. §1.821 through § 1.825 due to failure to use a sequence identifier for the EcoRI linker sequence on page 27 in paragraph [0077] and the requirement for submission of a computer readable sequence listing, a paper sequence listing, and a statement that the content of the paper and computer readable copies are the same and include no new matter to introduce the sequence of the EcoRI linker.

M.P.E.P 2422.01 *Definitions of Nucleotide and/or Amino Acids for Purpose of Sequence Rules – 2400 Biotechnology*, defines the sequences to which 37 CFR 1.821 through 1.825 apply as follows: "Nucleotide and/or Amino acid sequences as used in 37 CFR 1.821 through 1.825 are interpreted to mean an unbranched sequence of four or more amino acids or an unbranched sequence of ten or more nucleotides." In the present case, the Eco RI linker sequence on page 27 of the application contains only eight nucleotides. Applicants respectfully submit that M.P.E.P 2422.01 excludes such short sequences from the requirements under 37 C.F.R. §1.821 through § 1.825. With regard to the sequences designated as SEQ ID NO:1 and SEQ ID NO:2, Applicants submit herewith a Communication requesting transfer of previously filed sequence information to the instant application as requested by the Examiner. Accordingly, Applicants

respectfully request reconsideration and withdrawal of the requirement for Applicants to provide a sequence identifier for the EcoRI linker sequence and to submit a computer readable sequence listing, a paper sequence listing, and a statement that the content of the paper and computer readable copies are the same and include no new matter for the present application.

Drawings

The Office Action further indicates that the corrected drawings received on August 27, 2002 are acceptable, but are objected to for being on paper of different size than that of the originally filed drawings. The Examiner requests resubmission of the corrected drawings on 8 ¼ in. by 11 5/8 in. paper. To overcome the objection, Applicants attach hereto are copies of Figures 6-8, 14 and 15 on 8 ¼ in. by 11 5/8 in. paper. Accordingly, Applicants respectfully request reconsideration and withdrawal of the objection to the drawings.

The Objection to the Claims

Claim 48 is objected to for an alleged informality in omission of a phrase. To overcome the objection, claim 48 has been amended by the present communication to insert the phrase "library in" prior to "*E. coli*" as suggested by the Examiner. In addition, although claim 55 is not objected to, the Examiner has questioned whether Applicants intended that the substrate in claim 54 should be "*for* a thioesterase, rather than the substrate itself *is* a thioesterase. Applicants have amended claim 54 to clarify that the substrate is a substrate "for a thioesterase." In view of the amendments to claims 48 and 54, Applicants respectfully request reconsideration and withdrawal of the objection to the claims.

The Rejection Under 35 U.S.C. § 102 (e)

Applicants respectfully traverse the rejection of claims 1-4, 6-10, 16, 17-19, 23-27, 29, 30, 32-26, and 39-45 under 35 U.S.C. § 102(e) over U.S. Patent No. 5,824,485 to Thompson et al. (hereinafter "the '485 patent"). Applicants respectfully submit that the invention methods for identifying bioactivities or biomolecules, as recited by amended claims 1 and 26 distinguish over the disclosure of Thompson, at least by reciting:

providing an environmental gene library containing a plurality of clones, wherein DNA for generating the library is naturally occurring and obtained from ~~more than one~~ a mixed population of uncultured organisms; encapsulating a bioactive substrate and at least one clone of the library in a gel microdroplet, wherein an bioactivity or biomolecule produced by the clone is detectable by a change in fluorescence of the substrate prior to contacting with the at least one clone as compared to after the contacting; and screening the microdroplet with an assay or an analyzer that detects the presence therein of the change in the substrate, wherein the change indicates the identity of the bioactivity or biomolecule.

Thus, Applicants' presently claimed invention, as defined by amended claim 1, 27 and 28 is not the purposeful creation of novel activities or pathways by combinatorial techniques, but rather expression cloning of DNA derived from a mixture of uncultivated organisms to produce libraries that contain naturally occurring activities or gene clusters or pathways or genes as found in nature, without manipulation. It can be envisioned that once Applicants have cloned DNA producing such activities or pathways as they occur naturally in organisms in the environment, such molecules could be further manipulated by substituting genes or portions thereof from other species or strains using combinatorial methods. Thus, Applicants reiterate that the claimed invention is not directed to a "combinatorial library," but rather recites screening environmental

libraries containing sequences that are naturally occurring and which have not been rearranged or recombined in a laboratory setting for the purpose of creating new, combinatorially produced, pathways.

By contrast, Applicants respectfully submit that the '485 patent fails to describe each and every element of Applicant's methods for enriching for target DNA sequences containing at least a partial coding region for at least one specified activity in a DNA sample, as defined by amended claims 1, 27 and 28. Instead, the '485 patent describes *combinatorial* gene expression libraries constructed by stochastic genetic manipulation from genetic material of organisms that are known or are prospective sources of drugs. For example, the '485 patent teaches that individual genes from different species can be concatenated in a way that is predetermined so as to produce a potentially novel, but non-naturally occurring pathway. In addition, the '485 patent teaches cloning genes, at least one of which is a known gene, from several different organisms into a single host cell, with the result being the formation of potentially new pathways. For example, in the '485 patent, a single host cell might contain gene A, which is a known gene, from organism A, gene B from organism B and gene C from organism C, thereby producing a novel metabolic pathway encoded by genes combined from various organisms.

Applicants provide extrinsic evidence in support of the meaning of "combinatorial" as used in the '485 teaching to distinguish from Applicants' teaching and claims directed to naturally occurring DNA encoding activities of interest, including operons. Exhibit A is a print out from an internet site which includes a description of Neugenesis' combinatorial biology technology, which creates "combinatorial panels of heavy and light chains of a heteromeric protein and to build libraries of diverse, new, fully assembled proteins. Variants of each subunit gene are generated within the host by Neugenesis' proprietary technology."

(http://www.neugenesis.com/) Clearly, Applicants' claims are not directed to combinatorial approaches to identifying enzyme activities encoded by naturally occurring gene clusters, since Applicants are not manipulating the DNA to generate variants.

Exhibit B is a printout from the internet site of the Koide Group, from University of Pittsburgh (http://www.pitt.edu/~sparano/group/). As you will note, the study of Natural Products is separate and distinct from the study of Combinatorial Libraries. Exhibit B provides a glossary of terms used in Medicinal Chemistry. On page 4, the term combinatorial synthesis is described as "...combining sets of building blocks" e.g., ligating together individual genes of a gene cluster.

Further, Applicant submits that the "support" for libraries in the priority documents of the '485 patent, do not anticipate Applicants claimed invention. The Examiner relies on the '485 text for support for arguments presented in the office action, however, Applicants do not believe that the 08/427,348 or 08/427,244 documents, filed April 24, 1995, provide support for anything other than combinatorial libraries and certainly do not constitute prior art for Applicant's claimed "environmental expression libraries". For example, the section page 21 of the '244 document, is entitled "Library Design" and line 2 states that a number of "design strategies" can be used for library assembly. The Applicants' claimed invention is not directed to "designed" libraries; Applicants' libraries are produced randomly from nature. The first library in the '244 patent application, described on lines 4-11 of page 21, are directed to "designed" libraries containing known (previously cloned) and unknown genes. Again, Applicants' libraries are not "designed" at all. The Complex Combinatorial Libraries described on lines 12-18 of page 21 of the '244 document are "designed" by piecing together "smaller DNA fragments collected from each

species to be put into the library". Fragments are then concatenated to produce new pathways. This "complex combinatorial" library "design" is not the same as Applicants' invention. Applicants submit that all of this evidence supports Applicants' prior arguments distinguishing Applicants' claimed invention from the combinatorial methods described in the '485 patent.

Moreover, Applicants respectfully submit that the invention screening methods defined by amended claim 26 further distinguish over the disclosure of Thompson at least by reciting in step c) "inserting a polynucleotide into the clones of the library, wherein the polynucleotide encodes a bioactive protein substrate, wherein a fluorescence change in the substrate is detectable in the presence of a bioactivity or biomolecule." In the Office Action, the Examiner acknowledges that Thompson is silent regarding inserting a polynucleotide into the clones, wherein the polynucleotide encodes a bioactive substrate that undergoes a fluorescence change that is detected in the presence of an enzymatic bioactivity or biomolecule in the clone (Office Action, page 8). Applicants respectfully submit, therefore, that Thompson further fails to disclose each and every element of independent claim 26 (and all claims dependent thereon) as would be required to establish anticipation under 35 U.S.C. 102(e).

Accordingly, Applicants respectfully submit that Thompson fails to disclose each and every element of independent claims 1 and 26 (and all claims dependent thereon) as would be required to establish anticipation under 35 U.S.C. 102(e).

The Rejection under 35 U.S.C. § 103

Applicants respectfully traverse the rejection of claims 1-6, 8-19, and 24-26 under 35 U.S.C. § 103 as allegedly being unpatentable over the '485 patent as applied above and in view

of Short (U.S. Patent No. 5,958,672; hereinafter "Short"). The deficiencies of the '485 patent described above for disclosing the invention methods apply equally and are incorporated here. In addition, with regard to Short, Applicants submit herewith a Declaration under 37 C.F.R. §1.132 executed by Jay M. Short, President, Chief Executive Officer and Chief Technology Officer of Diversa Corporation declaring that the present application and U.S. Patent No. 5,958,672 are commonly owned by Diversa Corporation, and were co-owned at the time of the respective inventions, as evidenced by Assignments recorded at the U.S. Patent and Trademark Office. In view of this Declaration, Applicants submit that U.S. Patent No. 5,958,672 is not available as a reference under 35 U.S.C. § 102(e)/103 as amended by H.R. 2215 (Technical Amendment Act). Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection under 35 U.S.C. § 103 over the combined disclosures of Thompson and Short.

The Rejection under 35 U.S.C. § 103(a)

To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all of the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). The mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. *In re Mills*, 916 F.2d 680, 16 USPQ2d 1430 (Fed. Cir. 1990).

A. Applicants respectfully traverse the rejection of claims 1-4, 6-15, 16-19, 23-27, 29, 30, and 32-45 under 35 U.S.C. § 103(a) over Thompson as applied in the rejection under 35 U.S.C. § 102(e) above and further in view of Plovins et al. (*App. Environ. Microbiology* (1994) 60:4638-4641; hereinafter "Plovins") and Zhang et al. (*FASEB J.* (1991) 5:3108-3113; hereinafter "Zhang"). Applicants respectfully submit that the invention methods for identifying bioactivities or biomolecules using high throughput screening of nucleic acid, as defined by amended claims 1 and 26, distinguish over the combined disclosures of Thompson, Plovins and Zhang for the same reasons as discussed above with reference to Thompson. The deficiencies of Thompson for disclosing the invention methods for identifying bioactivities or biomolecules using high throughput screening of nucleic acid discussed above apply equally here. In particular, Thompson fails to disclose making and screening an environmental gene library from a mixed population of uncultured organisms. In addition, Applicants submit that Thompson does not suggest modifying the disclosed methods along the lines of Applicants' invention.

Plovins fails to cure these deficiencies in Thompson. The Examiner relies upon Plovins as disclosing use of FDG as well as C_{12}FDG as substrates in animal, bacterial and yeast cells. However, Plovins fails to disclose co-encapsulation of FDG or C_{12}FDG with DNA in a gel microdroplet for screening of whole microdroplets. Similarly, Plovins fails to disclose insertion of a polynucleotide encoding a substrate directly into an environmental library of naturally occurring DNA clones obtained from a mixed population of uncultured organisms wherein a bioactivity or biomolecule produced by the clone changes the substrate, indicating the presence and identity of the bioactivity or biomolecule in the clone. Thus, Applicants respectfully submit that the combined disclosures of Thompson and Plovins fail to teach or suggest the invention methods for identifying bioactivities or biomolecules using high throughput screening, as defined by amended claims 1 and 26.

Like Plovins, Zhang also fails to cure the deficiencies of Thompson for teaching or suggesting the invention methods for identifying bioactivities or biomolecules using high throughput screening, as defined by amended claims 1 and 26. The Examiner relies upon Zhang for disclosure of the development of lipophilic, fluorogenic substrates derived from FDG, such as FDG having an added lipophilic tail, to enable the substrate to pass through the cellular membrane. However, Zhang fails to disclose co-encapsulation of such a substrate with DNA from an environmental library containing naturally occurring DNA in a gel microdroplet for detection of microdroplets wherein a change in the substrate signals the presence and identity of a clone that interacts with the substrate to cause the change. Similarly, Zhang fails to disclose or suggest insertion into a library clone of a bioactive substrate for identification of clones that cause a change in the substrate, thereby identifying the identity of the substrate. In view of the failure of either Plovins or Zhang to cure the above-described deficiencies of Thompson for suggesting the invention methods, Applicants respectfully submit that the combined disclosures of Thompson, Plovins and Zhang are not sufficient to teach or suggest the present invention under 35 U.S.C. § 103, and reconsideration and withdrawal of the rejection is respectfully requested.

B. Applicants respectfully traverse the rejection of claims 1-4, 6-10, 16-19, 23-30, 32-36, 39-45 and 51-54 under 35 U.S.C. § 103(a) for allegedly being unpatentable over the combined disclosures of Thompson in view of Tsien et al. (U.S. Patent No. 5,981,200; hereinafter "Tsien"). Applicants respectfully submit that the invention methods for identifying bioactivities or biomolecules using high throughput screening of nucleic acid, as defined by amended claims 1 and 26, distinguish over the combined disclosures of Thompson and Tsien for the same reasons as discussed above with reference to Thompson. The deficiencies of Thompson for disclosing

the invention methods for identifying bioactivities or biomolecules using high throughput screening of nucleic acid discussed above apply equally here.

Tsien fails to cure these deficiencies in Thompson for suggesting the invention methods for screening environmental libraries. The Examiner relies upon Tsien as disclosing a fusion protein comprising an amino acid linker which is an enzyme substrate flanked by fluorescent proteins, such as green fluorescent protein, optionally encoded by a polynucleotide that can be expressed in recombinant cells to screen for enzyme activities. However, Tsien fails to disclose or suggest co-encapsulation of such a fusion protein within a micro-environment containing naturally occurring DNA obtained from a mixed population of uncultured organisms for the purpose of screening a library of uncultivated organisms.

In view of the failure of Tsien to cure the above-described deficiencies of Thompson for suggesting the invention methods, Applicants respectfully submit that the combined disclosures of Thompson and Tsien are not sufficient to teach or suggest the present invention under 35 U.S.C. § 103, and reconsideration and withdrawal of the rejection is respectfully requested.

The Rejection Under 35 U.S.C. § 112, First Paragraph

Applicants respectfully traverse the rejection of claim 28 under 35 U.S.C. § 112, First Paragraph, as containing subject matter allegedly lacking sufficient description in the Specification. In particular, the Examiner asserts that claim 28 pertains to an assay method wherein a nucleic acid substrate that is encoded by a polynucleotide has a detectable change upon exposure to an enzyme (Office Action, page 10). In addition, the Examiner asserts that the Specification provides sufficient support only for fluorescent protein substrates. However, it appears that the Examiner intended to refer to claim 26, which contains language the Examiner

has construed as claiming a polynucleotide substrate, while claim 28, pertains to particular types of enzymes whose activity can be detected using the invention methods. To clarify claim 26 as well as overcome the rejection, claim 26 has been amended to recite: "inserting a polynucleotide into the clones of the normalized library, wherein the polynucleotide encodes a bioactive protein substrate, wherein a fluorescence change in the substrate is detectable in the presence of a bioactivity or biomolecule." Applicants submit that amended claim 26 clarifies that the polynucleotide encodes a protein substrate that undergoes a change in fluorescence as a result of enzymatic action by the clones in the normalized library with the protein substrate. The Examiner acknowledges that the Specification provides adequate description for claim 26 to recite "fluorescence for the bioactive substrate that is encoded by a polynucleotide" (Office Action, page 10). Accordingly Applicants respectfully request reconsideration and withdrawal of the rejection of claim 28 under 35 U.S.C. § 112, First Paragraph.

Applicants respectfully traverse the rejection of claim 55 under 35 U.S.C. § 112, First Paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to make and/or use the invention. In particular, the Examiner asserts that claim 55, which is drawn to claim 54 wherein the substrate is a thioesterase, would require one of skill in the art to undertake a large quantity of trial and error experimentation to determine what bioactivity or biomolecule specifically targets thioesterase as a substrate and what fragment of a thioesterase is sufficient to be a substrate while not precluding the fluorescence change associated with the cleavage of the fusion protein. Applicants submit that amended claim 55 clarifies that the substrate is a substrate *for* a thioesterase, as opposed to the substrate itself *being* a thioesterase. Therefore, one of skill in the art would not be required to undertake a large quantity of trial and error experimentation to make and/or use the invention.

Accordingly Applicants respectfully request reconsideration and withdrawal of the rejection of claim 55 under 35 U.S.C. § 112, First Paragraph.

The Rejection Under 35 U.S.C. § 112, Second Paragraph

Applicants respectfully traverse the rejection of claims 1-25 and 39-41 under 35 U.S.C. § 112, Second Paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Applicants apologize for the omission of the intended amendment to claim 1 in the previous response.

Claim 1 is rejected for allegedly insufficient antecedent basis for the limitation "the change". The Examiner further maintains that inconsistent terminology (i.e., use of both "change" and "difference") renders the metes and bounds of the claim uncertain. To provide internal consistency of terminology and provide proper antecedent basis, claim 1 has been amended to substitute "change" for "difference" in step b).

Claims 13 and 39 are rejected for allegedly being vague and indefinite. The Examiner asserts that it is unclear what "samples" are being referred to because claim 1 and claim 27 do not recite any samples. To clarify the subject matter that Applicants regard as the invention, claims 13 and 39 have been amended to substitute "the clones" for "samples".

Accordingly Applicants respectfully request reconsideration and withdrawal of the rejection of claims 1-25 and 39-41 under 35 U.S.C. § 112, Second Paragraph.

In view of the above amendments and remarks, Applicants respectfully submit that all objections and rejections have been overcome and allowance of the pending claims is

respectfully requested. If the Examiner would like to discuss any of the issues raised in the Office Action, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

Respectfully submitted,

Date: April 17, 2003

Lisa A. Haile, J.D., Ph.D.
Registration No.: 38,347
Telephone: (858) 677-1456
Facsimile: (858) 677-1465

USPTO CUSTOMER NUMBER 28213
GRAY CARY WARE & FREIDENRICH LLP
4365 Executive Drive, Suite 1100
San Diego, California 92121-2133

Enclosures: Exhibit A printout
Exhibit B glossary of terms
132 Declaration